A CAUSE OF RADIORESISTANCE IN RETINOBLASTOMA: PHOTORECEPTOR DIFFERENTIATION

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Reprinted from the Transactions
American Academy of Ophthalmology and Otolaryngology
SEPTEMBER - OCTOBER 1970
PRINTED IN U.S.A.

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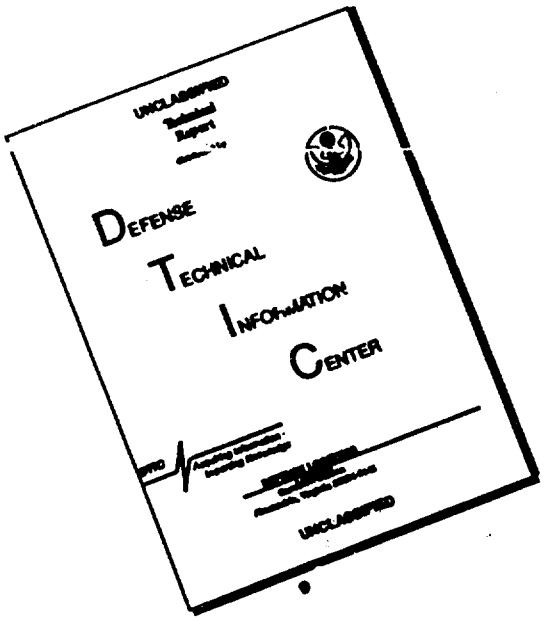

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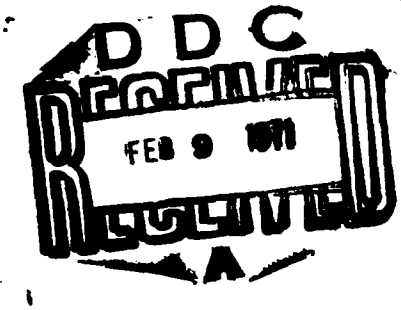

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RECENTLY a number of retinoblastomas were observed histologically to have areas of unusual differentiation in which a more mature cell type that differed from the Flexner-Wintersteiner rosettes was present.[5-7] These differentiated neoplastic cells showed convincing evidence by both light and electron microscopy that they had produced photoreceptor cell elements. In an earlier study,[6] tumors that had not received either radiotherapy or chemotherapy were examined to determine their morphologic and behavioral characteristics uninfluenced by treatment. In a small series of eight patients with an average follow-up period of eight years, there was no mortality. Even though the data suggest that these neoplasms are relatively benign, their biologic behavior remains uncertain.

In order to gain additional information concerning their behavior, the responses of these neoplasms to radiation were studied. The rationale for treating retinoblastomas with radiotherapy is based on the observation that, while undifferentiated tumor cells are very radiosensitive, the normal retina is comparatively resistant. This is in accord with the law of Bergonié and Tribondeau, which states, "radiosensitivity of cells varies inversely with their degree of differentiation and that the radiation sensitivity of cells is proportional to the duration of the period of mitotic and developmental activity they have yet to undergo."[3]

In this study, a series of enucleated eyes in which there were residual tumors following radiotherapy was examined. Our study was based on the premise that, if the law of Bergonié and Tribondeau should apply to tumors with photoreceptor differentiation, then these neoplasms should be relatively resistant to radiotherapy because of their advanced differentiation. We might then expect to find areas of photoreceptor differentiation in a larger proportion of

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This study was supported in part by a grant from The Seeing Eye, Inc., Morristown, NJ; by a Fight for Sight grant-in-aid (G-391) of the National Council to Combat Blindness, Inc., New York; and by Public Health Service research grants EY-00133 and EY-00397 and Public Health Service training grant EY-00032 from the National Eye Institute, US Public Health Service, Bethesda, Md.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Presented at the Seventy-fourth Annual Session of the American Academy of Ophthalmology and Otolaryngology, Chicago, Oct 12-16, 1969.

radioresistant tumors than in those that had not been treated with radiotherapy.

METHODS

Twenty-two retinoblastomas on file in the Registry of Ophthalmic Pathology and 32 on file at the Eye Institute, Columbia - Presbyterian Medical Center, were studied histopathologically. All eyes had been given radiation therapy for retinoblastoma before enucleation. The tumors on file in the Registry of Ophthalmic Pathology were from those eyes that had been treated in various centers throughout the world with no uniformity of method. The dosage of radiation varied from 3,000 r to 15,000 r, and the source of radiation ranged from radioactive cobalt to orthovoltage roentgen-rays. The treatment in one case had been supplemented with photocoagulation and in two cases with triethylene melamine. The eyes from the Columbia-Presbyterian Medical Center had been enucleated during the period, 1962-1967, after having been treated with one or two courses of 3,500 r to 4,000 r from the betatron. This treatment in some cases had been supplemented with triethylene melamine and with photocoagulation.

RESULTS

Of the 54 retinoblastomas studied histopathologically, viable residual tumors were observed in 42. Seventeen of the residual tumors exhibited areas of photoreceptor differentiation.

Clinical Data

The clinical data concerning these 17 patients are summarized in the Table. With the exception of a single case, all patients whose tumors contained areas of photoreceptor differentiation had retinoblastomas in both eyes. However, only one eye from each patient was examined. Histopathologic study of the fellow eye was not carried out because the material was unavailable to us. Clinically, however, 12 of the fellow eyes had been determined to have a more advanced disease and had been enucleated before radiotherapy was begun on the eyes that we studied. Four patients had received radiation to both eyes, and three of them retained the fellow eye; only one of these patients had had enucleation of each eye following irradiation. No clinical information was available on 1 of the 17 patients.

The majority of the tumors had shown initial regression under radiation therapy, but all failed to regress entirely or to calcify completely. One eye did not respond in any discernible way and was enucleated. Nine eyes were enucleated later for presumed recurrence. In three patients, hemorrhage into the vitreous prevented an adequate examination by the clinician, and the suspicion of tumor growth led to enucleation. Extensive retinal detachment and incomplete regression of tumor led to enucleation in one eye. Three eyes were obtained at autopsy, but only one death was caused by spread of a tumor. One was considered an anesthetic death at the time of cosmetic orbital surgery. The third death followed administration of anesthesia for intra-arterial chemotherapy, the patient never having regained consciousness after the procedure.

Histopathology

Within the areas of photoreceptor differentiation, the presence of fleurettes[5-7] was the most characteristic feature in all 17 cases (Fig 1). The degree of differentiation of the fleurettes varied but, in general, it followed the pattern reported previously.[5,6] Small nuclei, abundant cytoplasm, and intercellular matrix were other criteria for identi-

TABLE
CLINICAL DATA ON 17 RETINOBLASTOMAS EXHIBITING PHOTORECEPTOR DIFFERENTIATION IN RESIDUAL TUMORS AFTER RADIOTHERAPY

DESCRIPTION	NO.
Sex:	
Male	11
Female	4
Unknown	2
Eyes involved with retinoblastoma:	
Both	16
Unknown	1
Family history of retinoblastoma:	
Positive	3
Negative	8
Unknown	6
Age of patient at recognition of signs:	
Birth to 1 yr	10
1 yr to 2 yr	2
Unknown	5
Presenting signs:	
Strabismus	5
Leukokoria	5
Poor vision	2
Unknown	5
Initial response to radiotherapy:	
Regressed	13
No response	1
Not clear	3
Clinical cause for enucleation:	
Apparent recurrence	9
Failure to respond to treatment	1
Vitreous hemorrhage and "?" recurrence	3
Retinal detachment and incomplete regression	1
Postmortem	
Death from tumor	1
Death from other causes	2
Follow-up periods:	
Under 1 yr	3
1 to 3 yr	6
Over 3 yr	4

fication of these differentiated tumor cells. Flexner - Wintersteiner rosettes were observed in residual nodules in two eyes; transitional forms between the Flexner - Wintersteiner rosettes and fleurettes were observed in these two cases and in one other case. In the great majority of the uncontrolled recurrent tumor growths, undifferentiated retinoblastoma cells predominated. The tumor nodules exhibiting photoreceptor differentiation showed three histopathologic patterns:

Group 1. In seven eyes, one of the residual tumor nodules consisted entirely of cells showing photoreceptor differentiation (Figs 2, 3, and 4). No undifferentiated retinoblastoma cells, extensive gliosis, or hemorrhages were noted in these tumor nodules. Calcium deposits were observed only occasionally. The tumor nodules were relatively avascular and when blood vessels were present, they had mildly thickened walls. Characteristically, the tumor nodules were small and endophytic.

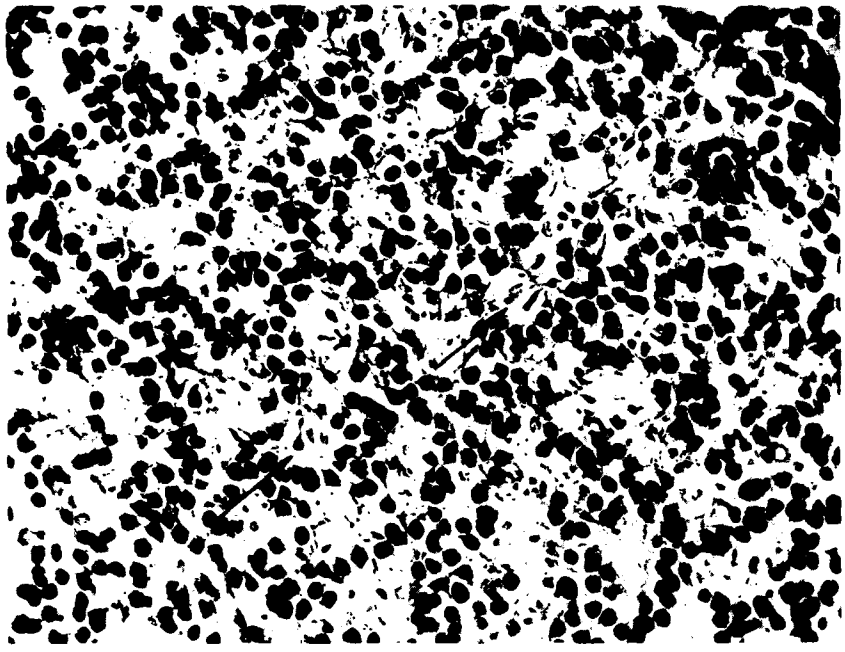

FIG 1—Irradiated retinoblastoma cells exhibiting photoreceptor differentiation. Tumor cells have mildly hyperchromatic nuclei, relatively abundant cytoplasm, and an intercellular matrix. Note presence of multiple well-differentiated fleurettes (*arrows*) (hematoxylin and eosin stain, × 300).

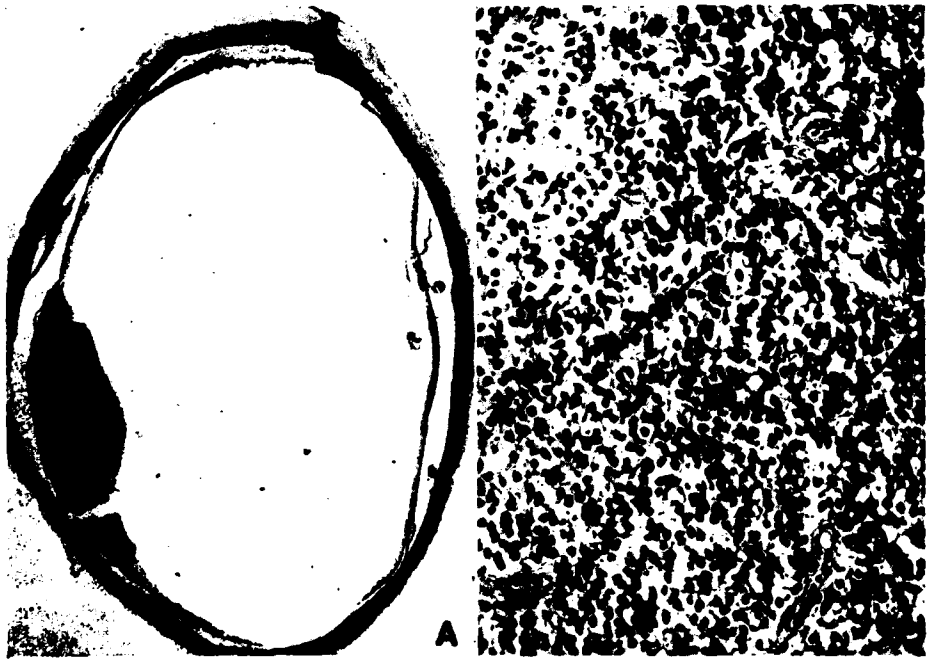

FIG 2—*A*, Retinoblastoma treated by radioactive cobalt and photocoagulation without response. Entire tumor consists of cells showing photoreceptor differentiation (group 1 pattern) (hematoxylin and eosin, × 5). *B*, Tumor cells at higher magnification show well-differentiated fleurettes *(arrows)* (hematoxylin and eosin, × 115).

FIG 3—*Center,* Residual retinoblastoma nodule after irradiation. Tumor is composed entirely of cells showing photoreceptor differentiation (group 1 pattern) (hematoxylin and eosin, × 20). *Upper left,* Inset shows fleurettes (*arrows*) in the tumor nodule at higher magnification (hematoxylin and eosin, × 395).

Four of the eyes in this group showed no other foci of undifferentiated tumor growth within the globe, and the nodule, consisting of cells exhibiting photoreceptor differentiation, was the only viable tumor that remained. It is noteworthy that the tumor in one of these patients was considered to be clinically controlled, but the patient died during cosmetic surgery. In one patient, the tumor failed to respond to irradiation and photocoagulation, and the eye was removed. The eye of the third patient was enucleated because of vitreous hemorrhage and suspicion of tumor activity. The tumor of the fourth patient was suspected of being active and was treated with intra-arterial triethylene melamine. The patient never recovered consciousness from general anesthesia.

Of the remaining three eyes in this group, independent foci of undifferentiated retinoblastoma cells were observed elsewhere in the same globe. The eyes were enucleated because of these regions of uncontrolled tumor growth.

Group 2. In this group of six patients, the residual tumors were buried within dense masses of glial cells (Fig 5). The blood vessels were thickened with hyaline degeneration, and vitreous hemorrhages were frequent. Calcification was seen occasionally. Deep within the glial masses, foci of tumor cells showing photoreceptor differentiation were found. No undifferentiated retinoblastoma cells could be found in four of the eyes. In three of the four eyes vitreous hemorrhage had occurred, and the eyes were

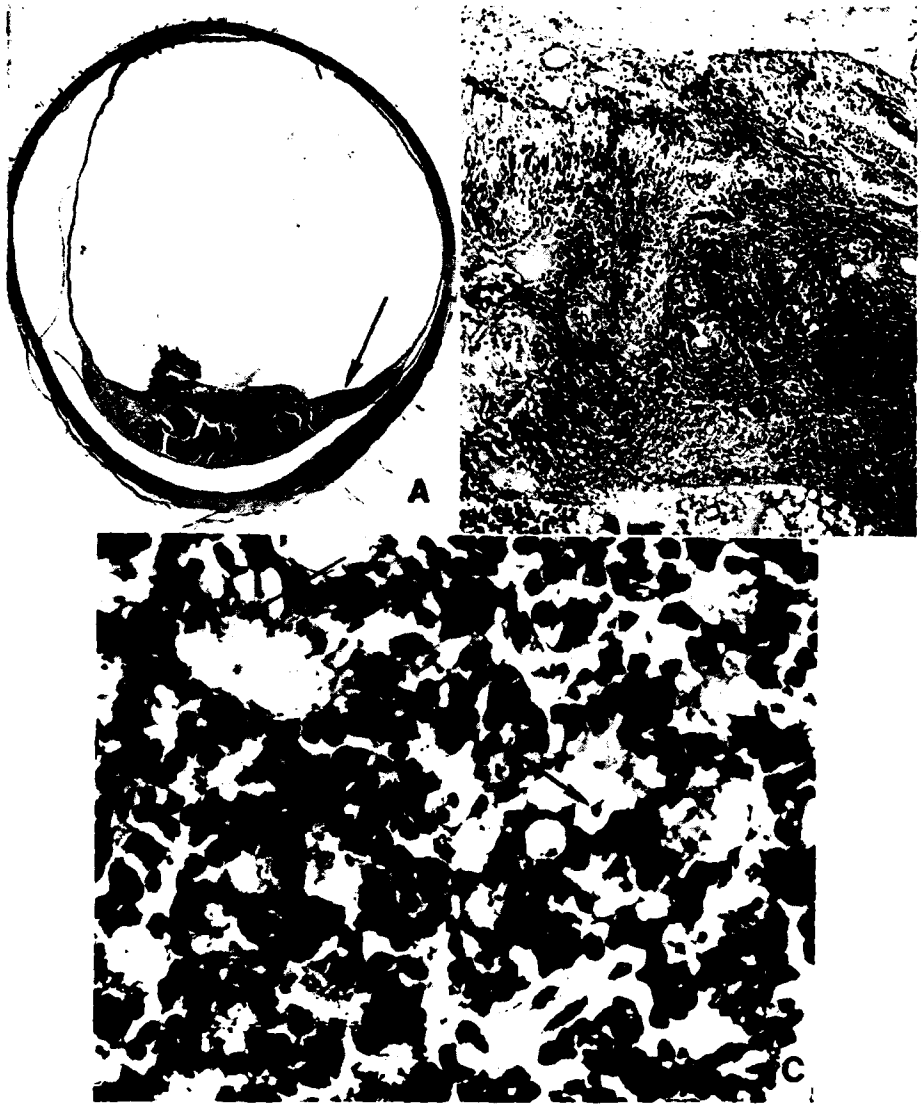

FIG 4—*A*, Irradiated retinoblastoma. Small residual tumor nodule (*arrow*) adjacent to disorganized and detached retina with extensive hemorrhage into the vitreous (hematoxylin and eosin, × 4). *B*, Retina exhibits loss of its normal architecture with much gliosis and focal hemorrhages, also extensive intraretinal and subretinal exudates (hematoxylin and eosin, × 19). *C*, Entire residual nodule shows photoreceptor differentiation; poorly differentiated fleurettes (*arrows*) present (hematoxylin and eosin, × 395).

enucleated because of the suspicion of tumor activity. An extensive retinal detachment and incomplete regression of tumor led to enucleation of the fourth eye.

The other two patients in this group had undifferentiated retinoblastoma cells in other independent foci of tumor growth. Tumor growth was correctly diagnosed, and the eyes were enucleated.

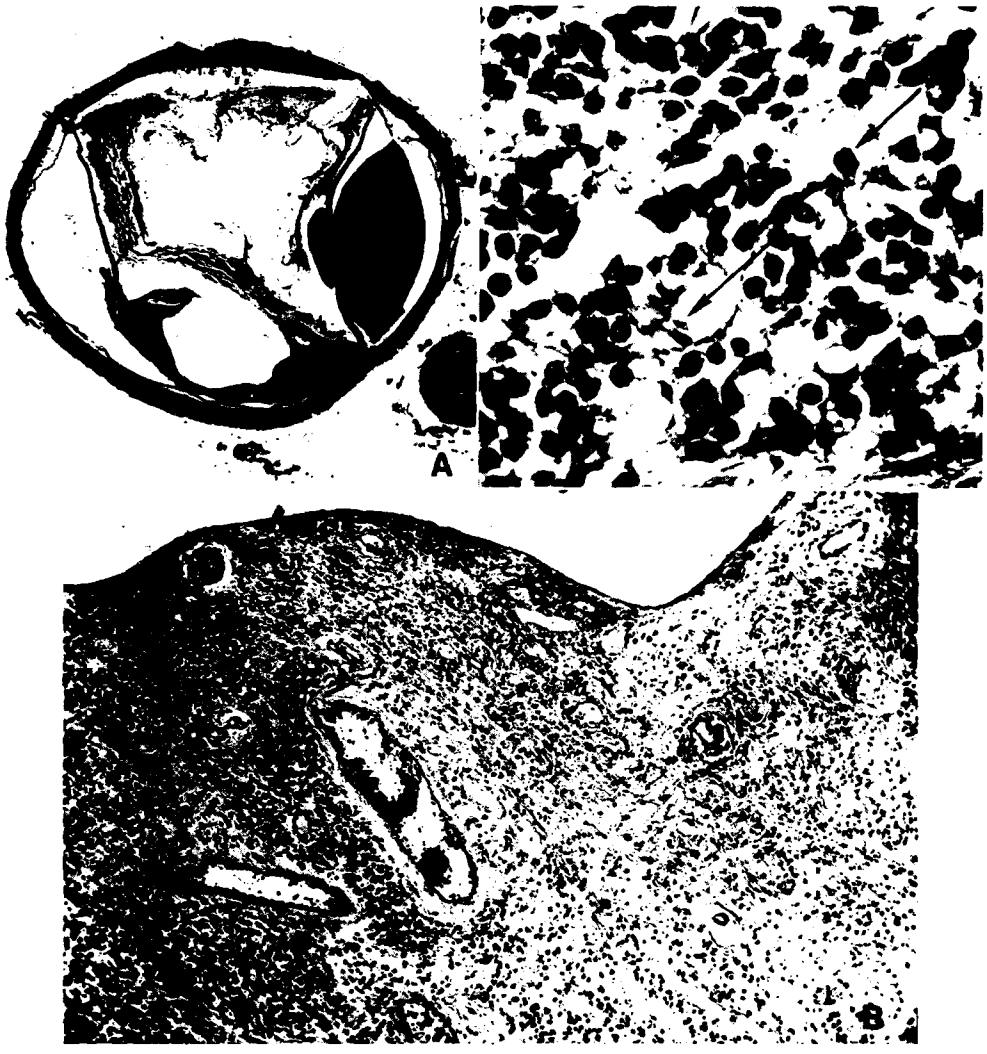

FIG 5—*A*, Persistent retinoblastoma following irradiation. Intravitreal hemorrhages and total retinal detachment (hematoxylin and eosin, × 3). *B*, Buried within masses of glial cells, foci of tumor cells (T) showing photoreceptor differentiation (group 2 pattern) (hematoxylin and eosin, × 60). *C*, Tumor cells showing photoreceptor differentiation *(arrows)* at higher magnification (hematoxylin and eosin, × 395).

Group 3. In this group of four patients, tumor cells with photoreceptor differentiation merged into areas of undifferentiated retinoblastoma cells within the same nodule (Figs 6 and 7). Demarcation between these two areas was not distinct. Tumor giant cells were observed. All four retinoblastomas showed initial regression with subsequent recurrence. The eyes were enucleated because of uncontrolled tumor growth. The parents of one of the patients, however, refused to permit enucleation. The patient died of intracranial extension of the tu-

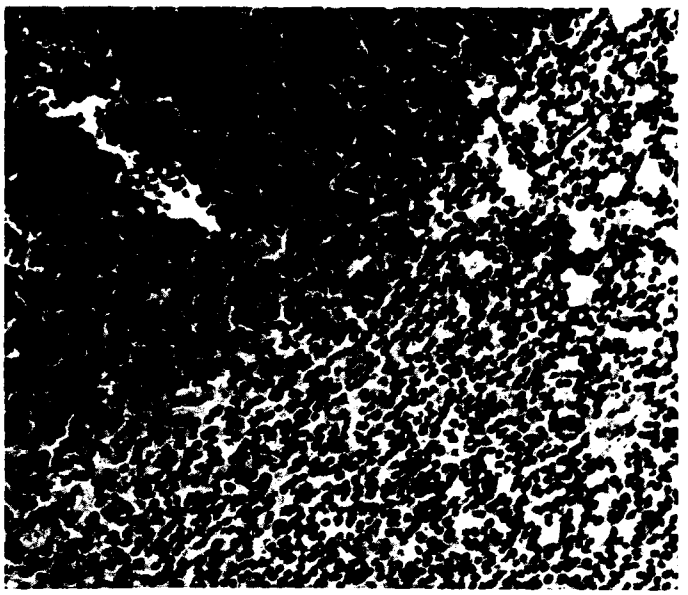

FIG 6—Retinoblastoma cells showing photoreceptor differentiation (P) merge with undifferentiated tumor cells (group 2 pattern); poorly differentiated fleurettes *(arrow)* in differentiated area (hematoxylin and eosin, × 168).

mor; the eye was obtained at autopsy. This was the only death caused by a tumor in this series of 17 cases in which photoreceptor differentiation was found.

Although the foregoing discussion and the grouping of lesions would seem to indicate that each eye contained only one nodule in which the tumor exhibited photoreceptor differentiation, actually there were three eyes (one in each of the three groups) that contained a second lesion showing photoreceptor differentiation. In each instance the second lesion was smaller and less conspicuous than the one that was used for the primary grouping. The second lesion in each instance belonged in group 1. Twenty lesions were found that exhibited photoreceptor differentiation in these 17 eyes—ten were in group 1, six in group 2, and four in group 3.

Ophthalmoscopic Characteristics of Photoreceptor Differentiation

We had detailed clinical descriptions before and after treatment that could be compared with the histopathologic sections for four tumor nodules from three patients. Two of these nodules were composed entirely of tumor cells exhibiting photoreceptor differentiation (group 1), while two nodules contained foci of photoreceptor differentiation buried in a glial mass (group 2). These nodules were observed to have regressed with radiation therapy and had become grayish in color and translucent. These persistent nodules were described as having a "fish flesh" appearance. One of these nodules had pigmentary degeneration around its base.

DISCUSSION

Of the 54 eyes with retinoblastomas that had been enucleated following ra-

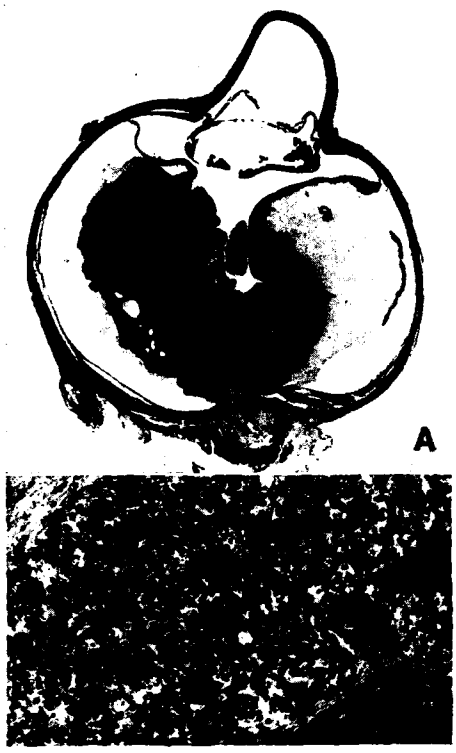

FIG 7—*A*, Tumor recurrence in previously irradiated retinoblastoma. Noteworthy is a nodule (P) within larger mass of undifferentiated tumor cells; photoreceptor differentiation (P) observed in nodule is shown at higher magnification in *B*, (hematoxylin and eosin, × 3). *B*, Well-differentiated fleurettes *(arrow)* are observed in differentiated area (hematoxylin and eosin, × 115).

diotherapy, viable residual neoplastic tissue was observed in 42 and 40% of these contained foci of photoreceptor differentiation. This frequency is significantly higher than that observed in untreated retinoblastoma (6%),[6] but it is undoubtedly lower than the true incidence of tumor cells showing photoreceptor differentiation, for no attempt was made to increase the yield of these differentiated tumors by preparing additional sections from the 25 tumors in which photoreceptor differentiation was not observed.

The simplest explanation for such a high incidence of photoreceptor differentiation in irradiated retinoblastomas is that these highly differentiated components are comparatively resistant to radiation and remain as residual nodules, while the undifferentiated retinoblastoma cells disintegrate under treatment. This explanation is consistent with the law of Bergonié and Tribondeau. It also appears to be consistent with the clinical observation of partial regression of these tumors with radiation therapy but of failure to achieve complete regression. On the other hand, the possibility cannot be ruled out that some of the undifferentiated retinoblastoma cells may mature and differentiate photoreceptor elements while being irradiated.

The persistence of tumor cells with photoreceptor differentiation in irradiated retinoblastomas suggests that these tumor cells behave as highly differentiated and mature cells. This consideration is in agreement with the morphologic characteristics of these tumor cells (Fig 1): The relatively small nuclei, abundant cytoplasm, and extracellular matrix suggest cellular maturity. The rarity of mitoses implies benignity. The presence of photoreceptor elements indicates an advanced degree of differentiation. Such cytologic impressions are further supported by the follow-up data. In the 13 patients in this series with an average follow-up period of three years, there was only 1 death from tumor. This fatality might have been avoided if enucleation had been permitted when the tumor recurred. In our previous series[6] of eight retinoblastomas treated by enucleation only, with an average follow-up period of eight years, there was no mortality.

Some additional support for our belief that these tumors carry a favorable prognosis is obtained from the literature. Reese[1] has observed viable tumor cells in residual tumors years after irradiation and postulated "suspended vi-

ability" of the tumor cells. Stallard[4] also noted that tumor cells could undergo a stage of "suspended activity." It is possible that these may be examples of tumors exhibiting photoreceptor differentiation.

The poor response of these tumors to irradiation, their cytologic features, and the follow-up data of two small series all point to the relative benignity of these neoplasms. Conservative management of such residual tumors would seem justifiable. It is possible, however, that such differentiated tumor cells may, under certain circumstances, become malignant again. The histopathologic pattern of the tumors in group 3 may reflect such changes (Figs 6 and 7).

The ophthalmoscopic appearance of tumor areas with photoreceptor differentiation is not entirely clear. In the four clinically well documented cases, the clinicians described the residual tumors as grayish, translucent, or "fish flesh" in appearance. Reese and colleagues[2] described three types of reaction of the retinoblastoma to irradiation; in one of them the partially regressed tumor residue "appears not as calcium but as a grayish-white, avascular mass smaller than the original tumor." Stallard[4] also observed that "sometimes the irradiated debris is grayish and is surmounted by dense white flecks with discrete edges." Two patients with such lesions remained asymptomatic under Stallard's care for eight years. It is possible that all such ophthalmoscopic descriptions apply to tumor areas with photoreceptor differentiation. More clinicohistopathologic correlations are necessary before more definitive conclusions can be drawn.

One of the fundamental principles in tumor pathology is that the better differentiated the tumor, the more innocent the tumor.[8] Ironically, one of the main principles in radiotherapy is that the better differentiated the tumor, the more radioresistant it will be.[3] The retinoblastoma cells exhibiting photoreceptor differentiation appear to behave according to principles of both pathology and radiotherapy.

SUMMARY

Forty-two eyes in which there were residual retinoblastomas following radiotherapy were examined histopathologically. Forty percent of the tumors showed areas of photoreceptor differentiation. Three histopathologic patterns were noted. Some tumor nodules consisted entirely of cells showing photoreceptor differentiation while other tumor nodules consisted of cells mingled with glial cells or undifferentiated retinoblastoma cells. Detailed clinical descriptions before and after radiation therapy were available on four of the tumor nodules. These tumor nodules were described as grayish, translucent, or "fish flesh" in appearance. In the follow up of 13 patients, only 1 tumor death was recorded. This study suggests that, while the tumors with photoreceptor differentiation are relatively benign histopathologically, they are comparatively resistant to radiotherapy.

Key Words: Retinoblastoma; photoreceptor differentiation; fleurette; radioresistance.

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DISCUSSION

ROBERT M. ELLSWORTH, MD, New York: Perhaps the most difficult problem in the treatment of retinoblastoma is the interpretation of regression patterns following radiation. It takes a good deal of experience to learn the significance of ophthalmoscopic changes, and that is why we urge our residents to see as many of these tumors as they possibly can. That is why, too, the same observer must follow these children serially. On a single fresh observation months after treatment, it is impossible to decide whether an eye is good, bad, or indifferent.

We have divided regression patterns into three groups: The first and easiest to interpret is called "cottage cheese" calcification. The glistening white material, which is precipitated deoxyribonucleic acid (DNA), binds calcium to form a radiopaque complex. This may well be the response of undifferentiated anaplastic cells exquisitely sensitive to radiation.

In a second pattern, no such glistening material is evident. The tumors shrink somewhat in size and lose the pink color of capillary injection. They remain elevated and the normal retinal vessels pass up over the surface. We have always considered these to be less sensitive tumor cells and it will be interesting to see if this type is composed largely of fleurettes when we have the opportunity, hopefully rare, to study them histologically.

The third pattern is a combination of the first two with a nidus of glistening deoxyribonucleic acid calcium (DNA-Ca), surrounded by gray translucent material, through which the choroidal markings may be dimly made out.

Dr. Tsukahara spent a year with us studying the response of retinoblastoma to radiation and reported an analysis of 150 cases in the Archives of Ophthalmology in June 1960. He concluded that well-differentiated tumors, that is, those in which rosettes comprised over half the mass had a slightly better prognosis and that well-differentiated tumors showed greater resistance to roentgen-rays. There was a vague suggestion that radiation altered the histologic character of tumor cells, not in the direction of differentiation, but toward a more anaplastic type. A most interesting finding was the similarity in the degree of differentiation in patients with both eyes affected. Should this be true, an appreciation of the histologic type in an enucleated eye may give us a valuable clue to the regression pattern we may expect to find in the fellow treated eye.

At any rate, Drs. Ts'o, Zimmerman, and Fine today, have given us some confidence that the translucent tumor remnants that persist so long in these eyes have very little growth potential.